The evolutionary history of Beringian *Smelowskia* (Brassicaceae) inferred from combined microsatellite and DNA sequence data

Tor Carlsen, Reidar Elven & Christian Brochmann

National Centre for Biosystematics, Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, 0318 Oslo, Norway
Author for correspondence: *Tor Carlsen, tor.carlsen@bio.uio.no*

Abstract We used the genus *Smelowskia*, which is distributed in Asia and North America and comprises both diploids and polyploids, as an example to address phylogeny, biogeography and polyploidization in the recently (ca. 3 million years ago) formed arctic biome with particular reference to the Beringian area. We combined data from high-resolution nuclear markers (seven SSR loci) with sequences from two nuclear regions (the low copy *RPA2* region and the multicopy nrITS region) and five plastid regions (*trnL*^{UAA} and *rps16* introns, *trnH*^{GUG}-*psbA*, *trnL*^{UAA}-*trnF*^{GAA}, and *5′prs12-rpl20* spacers). The different nuclear markers showed a congruent pattern that fits well with that observed in morphology and geography, while the plastid data showed some incongruence. Both sequence and SSR data support merging of *Smelowskia porsildii*, *S. spathulatifolia*, and *S. jurtzevii* into a single species (*S. porsildii*). An Asian origin of the Beringian taxa was inferred, resulting in two separate lineages of American-Beringian/American taxa. The SSR data confirmed polyploidy in several species, supporting the major role of this process in the evolution of the arctic flora.

Keywords Beringia; biogeography; phylogeny; polyploidization; *Smelowskia*

■ INTRODUCTION

The present-day Arctic is a young biome generated by a climatic shift in the late Tertiary (Lafontaine & Wood, 1988; Bennike & Böcher, 1990; Matthews & Ovenden, 1990; Murray, 1995; Lear & al., 2000; Jahren, 2007). Murray (1995) suggested that the arctic flora of today is composed of a mixture of survivors from the arctic Tertiary forest, Pleistocene immigrants from various mountain areas, and *in situ* evolved Pleistocene taxa. Many arctic species are probably of Pleistocene origin, as shown, e.g., in *Cerastium* L. (Scheen & al., 2004; Brysting & al., 2007) and *Draba* L. (Koch & Al-Shehbaz, 2002; Grundt & al., 2006; see Brochmann & Brysting, 2008 for a review).

The region called Beringia, encompassing the region from the Kolyma River in Northeast Russia to the Mackenzie River in Canada, has probably played a key role in the evolution of the arctic flora and has served as a major refugium during the Pleistocene (Murray, 1995; Weider & Hobæk, 2000; Abbott & Brochmann, 2003; Hewitt, 2004; Geml & al., 2006; Tkach & al., 2008). However, in spite of the importance of this region, detailed reconstructions of the history of Beringian plants and their ancestral lineages are still scarce. Here we selected the genus *Smelowskia* C.A. Mey., which in its widest sense (Al-Shehbaz & Warwick, 2006) comprises several Beringian taxa as well as taxa confined to Central Asia/Himalaya and non-Beringian North America (Fig. 1; Table 1) as a model to study origin and evolution of Beringian taxa. *Smelowskia* is a taxonomically complex genus with both diploids and polyploids (Al-Shehbaz & Warwick, 2006).

In a phylogeny inferred from ITS and *trnL* intron sequences (Warwick & al., 2004), *Smelowskia* formed a monophyletic group together with eight other small genera: *Ermania* Cham. ex Botch., *Gorodkovia* Botch. & Karav., *Hedinia* Ostenf., *Hediniopsis* Botch. & V.V. Petrovsky, *Melanidion* Greene, *Redowskia* Cham. & Schltdl., *Sinosophiopsis* Al-Shehbaz, and *Sophiopsis* O.E. Schulz. This was followed up in the revision by Al-Shehbaz & Warwick (2006), who included all nine genera in a widely circumscribed *Smelowskia*, expanding the number of species from 8–10 to 25. *Redowskia* is the oldest published name, but it is a rare Siberian endemic and not well known. The name *Smelowskia* was thus conserved at the Botanical Congress in Vienna 2005 (Al-Shehbaz, 2003; Brummitt, 2005). This has avoided many nomenclatural changes and kept the traditional naming in horticulture.

Within *Smelowskia* s.l., five of the nine formerly recognized genera formed a supported monophyletic group with little internal structure (figs. 2–5 in Warwick & al., 2004), including *Smelowskia* s.str., *Ermania*, *Gorodkovia*, *Melanidion*, and *Redowskia*. This group (henceforth named the '*Smelowskia* clade') is distributed in South and East Siberia, Russian Far East, Beringia, and Cordilleran North America (Fig. 1). The remaining four among the formerly recognized genera occur in the Central Asian mountains of Tian-Shan, Pamir, and western Himalaya (*Hedinia*, *Sophiopsis*, *Sinosophiopsis*) and in eastern Beringia (Chukotka; *Hediniopsis*), and formed a paraphyletic group relative to the *Smelowskia* clade. In this study, we attempt to resolve the relationships within the *Smelowskia* clade as defined above.

There has also been extensive disagreement as to the delimitation of species within the *Smelowskia* clade. *Smelowskia calycina* (Stephan) C.A. Mey. was treated by Drury and Rollins (1952) as a widespread, polymorphic species with five varieties (var. *americana* (Regel & Herder) W.H. Drury & Rollins, var. *media* W.H. Drury & Rollins, var. *calycina*, var. *porsildii* W.H. Drury & Rollins, and var. *integrifolia* (Seem.) Rollins). This has been the most common treatment in North

American floras, as opposed to Russian authors considering *S. calycina* as Central Asian and absent from North America (Velichkin, 1979; Ovchinnikova, 2004). Rydberg (1902) and Velichkin (1979) treated these five taxa as separate species. The variety *integrifolia* was given the new name *S. spathulatifolia* (Velichkin, 1974). Warwick & al. (2004) treated *S. spathulatifolia* as a synonym for *S. americana* (Regel & Herder) Rydb., with a note that the taxon in Velichkin's delimitation includes material that belongs to *S. porsildii* W.H. Drury & Rollins) Jurtsev. However, Al-Shehbaz & Warwick (2006) suggested that *S. spathulatifolia* should be merged with *S. porsildii* into one highly variable species, but they emphasized a need for further studies. Based on a set of characters, including the shape of petals, stigma, and valves, Velichkin (1979) also described the new species *S. jurtzevii* and noted its close relationship to both *S. spathulatifolia* and *S. porsildii*. This taxon was treated as conspecific with *S. porsildii* by Czerepanov (1995) and Al-Shehbaz & Warwick (2006). *Smelowskia* (*Melanidion*) *borealis* (Greene) W.H. Drury & Rollins was treated as a species with four varieties in Drury & Rollins (1952). According to Warwick & al. (2004), one of these, *S. borealis* var. *jordalii*, is identical to *S. johnsonii*, described as a new species by Mulligan (2001).

As an initial framework for this study, we used the treatment of Al-Shehbaz & Warwick (2006), except that we treated *S. johnsonii* G.A. Mulligan as *S. borealis* (Greene) W.H. Drury & Rollins var. *jordalii* W.H. Drury & Rollins and thus recognized thirteen species within the *Smelowskia* clade (Fig. 1; Table 1). *Smelowskia alba* (Pall.) Regel, *S. bifurcata* (Ledeb.) Botsch and *S. calycina* occur in a belt from Lake Balkash through the Altai Mountains to Lake Bajkal, and *S. alba* is also found northwards along the Lena River to its delta. *Smelowskia americana* is the most widespread North American species found in the non-Beringian Rocky Mountains and in the Cascade Mountains of Canada and the United States, i.e., south of the Cordilleran and Laurentide glaciations. Two of the former *Melanidion* species, *S. pyriformis* W.H. Drury & Rollins and *S. ovalis* M.E. Jones, have very restricted distributions:

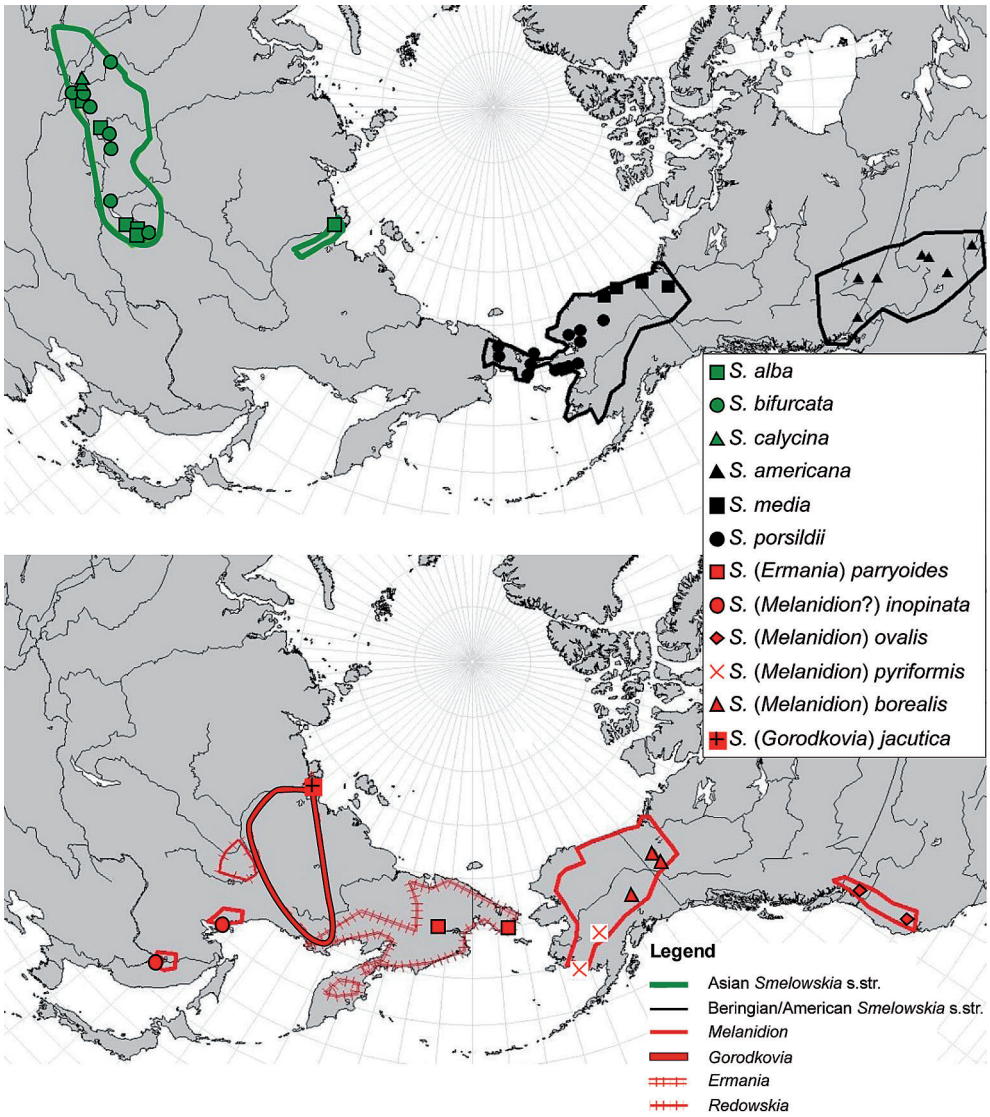

Fig. 1. Sampling sites and geographic distribution of the members of the *Smelowskia* clade.

Table 1. Taxa included in this study, with chromosome numbers,[a] approximate distribution area, and synonyms.

Taxon	2*n*	Area	Synonyms
Ingroup taxa[b]			
Smelowskia alba (Pall.) Regel	12	Central North Asia	*Sisymbrium album* Pall.
Smelowskia americana (Regel & Herder) Rydb.	12, 22	Western America	*Hutchinsia calycina* (Stephan) Desv. var. *americana* Regel & Herder *Smelowskia calycina* (Stephan) C.A. Mey. var. *americana* (Regel & Herder) W.H. Drury & Rollins
Smelowskia bifurcata (Ledeb.) Botsch.	12	Central Asia	*Hutchinsia bifurcata* Ledeb. *Smelowskia aspleniifolia* Turcz.
Smelowskia borealis (Greene) W.H. Drury & Rollins	12	American Beringia	*Melanidion boreale* Greene *Ermania borealis* (Greene) Hultén *Smelowskia borealis* (Greene) W.H. Drury & Rollins var. *jordalii* W.H. Drury & Rollins *Smelowskia borealis* (Greene) W.H. Drury & Rollins subsp. *jordalii* (W.H. Drury & Rollins) Cody *Smelowskia johnsonii* G.A. Mulligan
Smelowskia calycina (Stephan) C.A. Mey	12	Central Asia	*Lepidium calycinum* Stephan Willd.,
Smelowskia inopinata (Kom.) Kom.		Eastern Asia	*Hutchinsia inopinata* Kom.[c]
Smelowskia jacutica (Botsch. & Karav.) Al-Shehbaz & S.I. Warwick	12, 36	North north-eastern Asia	*Gorodkovia jacutica* Botsch. & Karav.
Smelowskia media (W.H. Drury & Rollins) Vielchkin	12	American Beringia	*Smelowskia calycina* (Stephan) C.A. Mey. var. *media* W.H. Drury & Rollins
Smelowskia ovalis M.E. Jones		Western America	
Smelowskia parryoides (Cham.) Polunin	12, 24	Asian Beringia	*Draba parryoides* Cham. *Ermania parryoides* (Cham.) Cham. *Christolea parryoides* (Cham.) N. Busch
Smelowskia porsildii (W.H. Drury & Rollins) Jurtsev	12, 18, 22, 24, 32	Amphi-Beringia	*Smelowskia calycina* (Stephan) C.A. Mey. var. *porsildii* W.H. Drury & Rollins *Smelowskia calycina* (Stephan) C.A. Mey. subsp. *integrifolia* (Seem.) Hultén var. *porsildii* (W.H. Drury & Rollins) Hultén *Smelowskia jurtzevii* Velichkin *Smelowskia spathulatifolia* Velichkin *Smelowskia calycina* (Stephan) C.A. Mey. var. *integrifolia* (Seem.) Rollins *Smelowskia calycina* (Stephan) C.A. Mey. subsp. *integrifolia* (Seem.) Hultén
Smelowskia pyriformis W.H. Drury & Rollins	12	American Beringia	
Smelowskia sophiifolia (Cham. & Schltdl.) Al-Shehbaz & S.I. Warwick		Central Asia	*Redowskia sophiifolia* Cham. & Schltdl.
Outgroup taxa:			
Smelowskia altaica (Pobed.) Botsch.		Central Asia	*Hedinia altaica* Pobed.,
Smelowskia czukotika (Botsch. & V.V. Petrovsky) Al-Shehbaz & S.I. Warwick	24	Asian Beringia	*Hediniopsis czukotica* Botsch. & V.V. Petrovsky, *Hedinia czukotic*a (Botsch. & V.V. Petrovsky) Jurtsev, Korobkov & Balandin,

[a] From (Drury & Rollins, 1952; Johnson & Packer, 1968; Packer, 1968; Yurtsev & Zhukova, 1972; Krogulevich, 1976; Zhukova & Petrovsky, 1977, 1980, 1984; Dawe & Murray, 1979; Zhukova, 1980; Murray & Kelso, 1997)
[b] Referred to as the *Smelowskia* clade in this text
[c] The genus *Melanidion* has partly been recognized in North America, but this species-group probably also includes *S. inopinata*

S. pyriformis is only found in the central mountains of Alaska whereas *S. ovalis* occurs in Washington, Oregon and the southernmost part of British Columbia. *Smelowskia borealis* is found in Alaska and in the Canadian districts of Yukon and the Northwest Territories as far as the Mackenzie River, whilst *S. media* (W.H. Drury & Rollins) Velichkin is found in Alaska, Yukon and the Northwest Territories. All these species are restricted to the unglaciated Beringian regions. The genus *Melanidion* has partly been recognized in North America, but this species group probably also includes *S. inopinata* (Kom.) Kom. that has a disjunct distribution and is found in the Khabarovsk and Okhotsk regions in the Russian Far East. *Smelowskia* (*Gorodkovia*) *jacutica* (Botsch. & Karav.) Al-Shehbaz & S.I. Warwick is found in the Okhotsk region and in the Verkhoyansk Mountains along the Lena River. *Smelowskia* (*Ermania*) *parryoides* (Cham.) Polunin and *S. porsildii* (including *S. jurtzevii* and *S. spathulatifolia*) are found in the Okhotsk region and on the Kamchatka and Chukchi peninsulas, with *S. porsildii* extending into Alaska.

Drury & Rollins (1952) assumed the present-day distribution of *Smelowskia* s.str. and *Melanidion* to result from fragmentation of an earlier continuous distribution throughout Siberia and North America. They also stated that the most probable place of origin is in North America with a later expansion into Siberia and the Altai mountains. Their assumption was based on the present distribution with no representatives found west of the Ural Mountains, and that more species occur in North America than in Siberia. Thus, they assumed that an Asian origin and a subsequent eastwards spread into and speciation in North America to be unlikely. However, by including Asian taxa, Warwick & al. (2004) found that central Asia was the most likely centre of origin for *Smelowskia* s.str.

Polyploidization events are frequent and play a major role in the evolution of the arctic flora (Scheen & al., 2002; Brochmann & al., 2004; Brysting & al., 2004, 2007; Popp & al., 2005; Jørgensen & al., 2006). The *Smelowskia* clade conforms to this trend, which may provide an explanation for the difficulties in species delimitation in this group. The basic chromosome number in *Smelowskia* is $x = 6$, with exclusively diploid counts of $2n = 12$ in *S. alba*, *S. bifurcata*, *S. calycina*, and *S. media* (*Smelowskia* s.str.) and in *S. borealis* and *S. pyriformis* (Drury & Rollins, 1952; Yurtsev & Zhukova, 1972; Krogulevich, 1976; Dawe & Murray, 1979; Zhukova & Petrovsky, 1980, 1984). Multiple cytotypes including diploids, pointing to frequent and recurrent polyploidizations, have been reported for four species: *S. americana* $2n = 12, 22$ (Drury & Rollins, 1952; Packer, 1968); *S. parryoides* $2n = 12, 24$ (Yurtsev & Zhukova, 1972; Zhukova & Petrovsky, 1977, 1980, 1984; Zhukova, 1980); *S. porsildii* $2n = 12, 18, 22, 24, 32$ (Johnson & Packer, 1968; Yurtsev & Zhukova, 1972; Yurtsev & al., 1975; Dawe & Murray, 1979; Zhukova & Petrovsky, 1984; Murray & Kelso, 1997); and *S. jacutica* $2n = 12, 36$ (Yurtsev & Zhukova, 1972, 1982).

As typically found in arctic plant groups, many of which may have evolved as late as during the Pleistocene (e.g., Grundt & al., 2004; Scheen & al., 2004), few phylogenetically informative characters were obtained from DNA sequences of *Smelowskia* in the study of Warwick & al. (2004). There is clearly a need for more high-resolution markers to address species delimitation and history of such groups. DNA fingerprinting such as RAPDs or AFLPs has been successfully used in combination with sequencing in some plant groups (e.g., Grundt & al., 2004; Eidesen & al., 2007b), but in order to obtain high-quality profiles, such markers require access to freshly collected material. This is often difficult to obtain for arctic-alpine groups occurring in widely separated, inaccessible areas, such as *Smelowskia*. Microsatellites represent an alternative type of high-resolution markers which can be obtained from herbarium material, and can be used to infer evolutionary relationships among closely related species where sequence variation is difficult to obtain (Goldstein & Pollock, 1997; Schlötterer, 2001; Jørgensen & al., 2008).

In this study, we address species delimitation and historical relationships in the arctic-alpine *Smelowskia* clade (as defined in this paper) using nuclear microsatellites in combination with plastid and low- and multicopy nuclear sequences from several regions (*trnL*^{UAA} intron, *trnL*^{UAA}-*trnF*^{GAA} intergenic spacer, *rps16* intron, *trnH*^{GUG}-*psbA* intergenic spacer, *5′prs12-rpl20* intergenic spacer, parts of the *RPA2* region, and ITS). In particular, we infer the biogeographic history of this North American/Beringian/Asian group, and address the usefulness of microsatellite markers for this kind of studies. Furthermore, we assess the validity of separating *S. spathulatifolia*, *S. porsildii*, and *S. jurtzevii* into distinct species.

■ MATERIALS AND METHODS

Leaf material was sampled from herbarium specimens held at ALA, CAN, DAO, LE, MHA, and O (see Table S1 in the Electronic Supplement to this article). In addition, some fresh leaf material was sampled and dried in silica gel in the field with vouchers deposited in the herbarium at the Natural History Museum, University of Oslo (O).

DNA was extracted using the DNeasy™ Plant Mini Kit or DNeasy™ Plant 96 Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. PCR amplification of nrITS was done with the primers ITS 4 and 5 (White & al., 1990). The *trnL*^{UAA} intron and the *trnL*^{UAA}-*trnF*^{GAA} intergenic spacer region were amplified with the primers c and f (Taberlet & al., 1991), the *rps16* intron with the primers of Oxelman & al. (1997) as modified by Shaw & al. (2005), the *trnH*^{GUG}-*psbA* intergenic spacer with the primers of Sang & al. (1997), and the *5′prs12-rpl20* intergenic spacer with the primers of Hamilton (1999). PCR reactions were performed with 30 cycles of 30 s at 94°C (first cycle 5 min), 30 s at 55°C, and 90 s at 72°C (last cycle 10 min). PCR products were purified with 10× diluted ExoSAP-IT® (USB Corporation, Cleveland, Ohio, U.S.A.) before cycle sequencing with 10× diluted BigDye (Applied Biosystems, Foster City, California, U.S.A.) in 25 cycles of 10 s at 96°C, 5 s at 50°C, and 240 s at 60°C and visualized on an ABI 3100 Sequencer (Applied Biosystems).

Parts of the *RPA2* region (corresponding to the 23rd intron in *Arabidopsis thaliana* (L.) Heynh.) from four species was amplified with first degenerated RNAP primers and then a nested

PCR with subunit-specific primers, as described by Popp & Oxelman (2004). This region is present in *Arabidopsis thaliana* and most *Silene* L. as a single copy unit (Popp & Oxelman, 2004; Popp & al., 2005). The PCR products were cloned using the kit TOPO TA Cloning Kit (Invitrogen, Carlsbad, U.S.A.) following the manufacturer's protocol except for using only half the reaction volumes. Individual colonies were subjected to PCR and sequenced. Taxon- and subunit-specific primers were designed and used in subsequent analyses for direct PCR and sequencing under the same conditions as described above. Smelowskia RPA2F: 5′-ATTGCCATGCTTTTGGAATC-3′, Smelowskia RPA2R: 5′-TCTCACACTGCAGCTCTACTCC-3′.

SSR loci were amplified with seven primers (Table 2). All primers but IS-17 were developed for *Arabidopsis thaliana*, but also amplify variable fragments within *Smelowskia* (Skrede & al., 2009). IS-17 was developed by searching the *A. thaliana* genome for microsatellite loci, and the primers are published in Table 2. The seven loci are distributed on all five chromosomes of *A. thaliana* (Table 2). All forward primers were given a tail with the M13 sequence (5′-CACGACGTTGTAAAACGAC-3′) (Schuelke, 2000), and used in combination with reverse primers and a third M13-primer dyed with FAM (MWG Biotech AG, Ebersberg, Germany), VIC (Applied Biosystems), NED (Applied Biosystems), or PET (Applied Biosystems). For the full protocol, see Skrede & al. (2009). The reactions were run for 5 min at 95°C, 35 cycles of the three steps 30 s at 94°C, 30 s at 51°C, and 45 s at 72°C, and a final hold of 20 min at 72°C. The PCR products were pooled, and 1 µL of product mixture (FAM : NED : PET : VIC = 2 : 3 : 3 : 2) was added 8.8 µL HiDi (formamide) and 0.2 µL GeneScan Liz 500 size standard (Applied Biosystems). The products were denatured for 5 min at 95°C and visualized on an ABI 3100 Sequencer (Applied Biosystems).

Sequences were edited in Sequencher v.4.1.4 (Gene Codes, Ann Arbor, Michigan, U.S.A.), and ambiguous positions were coded according to IUPAC standards. Available potential ingroup and outgroup sequences from GenBank were imported and included in the matrices. The sequences were subsequently aligned manually in BioEdit (Hall, 1999) and imported into TNT (Goloboff & al., 2008) through Genetool 2.0 (Biotools, Edmonton, Alberta, Canada). All sequences were submitted to GenBank, accession numbers EU489519–EU489556 and FJ972233–FJ972377.

Parsimony analyses of the sequence data were performed in TNT (Goloboff & al., 2008). Heuristic searches were performed with 1000 random addition sequences and TBR branch swapping, saving ten trees per replication. The resulting trees were swapped on with TBR saving up to 100,000 trees. Collapsing rule was set to minimum length = 0. Random seed was set to "time". Goodness of fit was calculated using CI, RI, RC according to Kluge & Farris (1969) and Farris (1989). Bremer support (Bremer, 1994) was calculated by producing 60,000 trees that were up to 6 steps longer, starting with saving 10,000 trees one step longer, and successively saving 10,000 tress of up to one step longer in 5 steps. Jackknife (Farris & al., 1996) and traditional bootstrap (Felsenstein, 1985) resampling studies were performed with 1000 replicates (10 random entry orders and 10 trees saved each repetition). Jackknifing was performed with 36% deletion. Both bootstrap and jackknife were performed with cut-off value of 50% and absolute frequencies as output.

Bayesian analyses of the ITS region were performed in MrBayes v.3.1.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) with the model SYM + gamma selected by hLRT, and the model GTR + gamma selected by AIC in MrModeltest v.2.2 (Nylander, 2004). The analysis was run for 6,000,000 generations in four chains sampling trees every 10,000th generation. Burn-in was set to 25%.

Arabidopsis and *Descurainia* were initially used as outgroups in the phylogenetic analyses. However, these taxa belong to other tribes in Brassicaceae (Al-Shehbaz & al., 2006) and have high sequence dissimilarity with the ingroup, which is not recommended for phylogenetic analyses (Felsenstein, 1978; Shavit & al., 2007). In these initial analyses, *Smelowskia altaica* (Pobed.) Botsch. and *Smelowskia czukotica* (Botsch. & V.V. Petrovsky) Al-Shehbaz & S.I. Warwick were resolved as the closest relatives to, and also distinct from the ingroup in this study, which is the *Smelowskia* clade as defined in the introduction. *Smelowskia altaica* and *S. czukotica* were thus suitable as outgroups.

Microsatellite profiles were sized and scored using GeneMapper v.3.7 (Applied Biosystems). Variation in ploidy level is problematic when scoring microsatellite loci. Different methods have been suggested in order to score and estimate distances among heterozygotes, but all make assumptions about the pattern of microsatellite evolution, which is uncertain (Harr & al.,

Table 2. Microsatellite primers used in this study. Chromosome number refers to the location in *Arabidopsis thaliana*.

Name	Chromosome number	Forward primer 5′–3′	Reverse primer 5′–3′	Number of alleles	Fragment length span
AthCTRI	5	TATCAACAGAAACGCACCGAG	CCACTTGTTTCTCTCTCTAG	14	117–196
SSL2	1	CATGTACTGGGATTCAGTGTCC	CGTCCTTTGTGTGGTTACACG	6	273–303
AthGAPAb	3	CACCATGGCTTCGGTTACTT	TCCTGAGAATTCAGTGAAACCC	3	157–161
IS-17	4	TTTGTTGCATCATCCTTTGC	GGCCTGCAATTTGAGACCTA	5	172–180
AthS0392	1	TTGGAGTTAGACACGGATCTG	GTTGATCGCAGCTTGATAAGC	3	155–159
nga129	5	TCAGGAGGAACTAAAGTGAGGG	CACACTGAAGATGGTCTTGAGG	2	173–185
nga1145	2	CCTTCACATCCAAAACCCAC	GCACATACCCACAACCAGAA	4	230–257

1998; Bruvo & al., 2004). Thus, we scored the microsatellites as dominant markers in this paper (allele present = 1, allele absent = 0, as in AFLP analyses). We hereby lose the information from the codominant properties of microsatellites, but gain a more reliable dataset (Jørgensen & al., 2008). We are aware of the potential problem with homoplasy when scoring microsatellites across several species, but because of our results were reasonable when compared to the results of the sequence analyses as well as to morphological and geographical information, we believe homoplasy plays a minor role in our dataset. The variation in the microsatellite dataset was visualized using principal coordinate analysis (PCO) in NTSYSpc v.2.02 (Rohlf, 1990) based on the similarity measure of Dice (1945). Due to high variation, the data matrix was successively split into smaller matrices according to the results of previous PCO analyses. A Bayesian approach using STRUCTURE v.2 (Pritchard & al., 2000) calculated a logarithmic probability for the data given a number of clusters and assigned the specimens to these clusters based on probability. The method may be applied to dominant markers under a no-admixture model, assuming no linkage between the loci (Pritchard & al., 2000). Ten replicates of each value of K (= the number of groups) were run for different selections of samples with a burn-in period of 100,000 and 1,000,000 iterations.

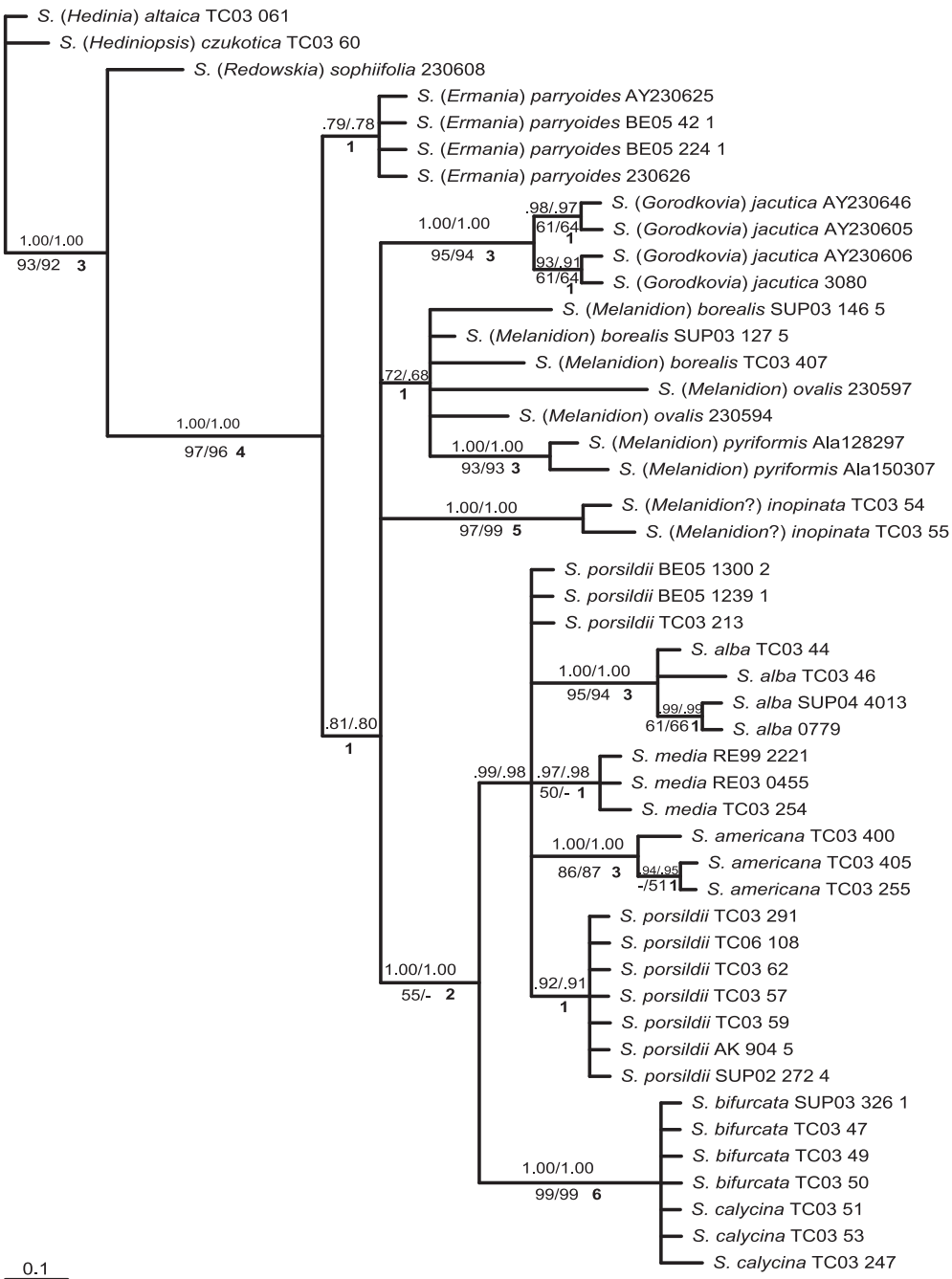

Fig. 2. ITS phylogram from a Bayesian analysis of the *Smelowskia* clade. Posterior probability values from two different models (SYMγ/GTRγ) are shown above branches. Jackknife and bootstrap support values from a parsimony analysis are shown below branches (JK/BS). Bremer support values are shown in bold below branches.

■ RESULTS

The matrix of ITS sequences had 595 characters of which 44 were potentially parsimony informative. None of the ambiguous positions coded according to IUPAC standards were potentially parsimony informative. We found 16 most parsimonious trees (MPTs) of length 84 from one island in the tree space. A Bayesian phylogram combining the results from the parsimony and Bayesian analyses of the nrITS sequences is presented in Fig. 2. Goodness of fit values were CI (consistency index) = 0.833, RI (retention index) = 0.926, and RC (rescaled consistency index) = 0.771. *Smelowskia sophiifolia* (Cham. & Schltdl.) Al-Shehbaz & S.I. Warwick was sister to the ingroup with a posterior probability (PP) of 1.0/1.0 (SYMγ/GTRγ), Bremer support (BR) of 4, Jackknife (JK) 97%, and Bootstrap (BS) 96%. *Smelowskia parryoides* was sister to all other taxa in the ingroup, but this sistergroup relationship only had a posterior probability of 0.81/0.80, BR of 1 and JK and BS below 50%. The tree was further divided into three (by parsimony analyses) or four (by Bayesian analyses) groups, with unresolved relationships. The first group consisted of only one species in both parsimony and Bayesian analyses: *Smelowskia jacutica* (PP 1.0/1.0, BR 3, JK 95%, BS 94%). The second group consisted of all the *Melanidion* species: *S. borealis*, *S. ovalis*, and *S. pyriformis* in the Bayesian analysis (PP 0.72/0.68, BR 1). The third group consisted of only one species: *Smelowskia inopinata* in the Bayesian analysis (PP 1.0/1.0, BR 5, JK 97%, BS 99%). The second and third group belonged to the same clade in the parsimony analysis. The fourth group comprised only species of *Smelowskia* s.str.: *S. alba*, *S. calycina*, *S. bifurcata*, *S. porsildii*, *S. americana*, and *S. media* (PP 1.0/1.0, BR 2, JK 55%).

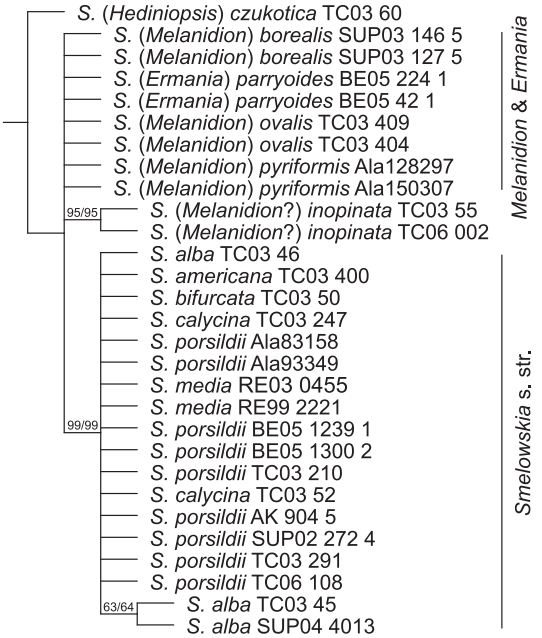

Fig. 3. Strict consensus tree of 8 most parsimonious trees from an analysis of *RPA2* sequences. Numbers above branches are jackknife/bootstrap support values.

The matrix of the *RPA2* sequences was 309 characters long, of which 15 were potentially parsimony informative. None of the ambiguous positions coded according to IUPAC standards were potentially parsimony informative. The *RPA2* parsimony analysis (Fig. 3) resulted in 8 MPTs of length 23 from one island in the tree-space. Goodness of fit values were CI = 1.00, RI = 1.00 and RC = 1.00. One well supported clade (JK = 99%, BS = 99%) was inferred, containing all taxa of *Smelowskia* s.str.

The combined matrix of plastid sequences had 2898 characters of which ~50 were potentially parsimony informative. The parsimony analysis of the plastid matrix (Fig. 4) resulted in 1365 MPTs of length 96 from one island in the tree-space. Goodness of fit values were CI = 0.83, RI = 0.93, and RC = 0.78. The tree had low resolution with one branch separating *S. alba*, *S. jacutica*, and *S. parryoides* from the rest of the ingroup (JK 60% and BS 60%). The other supported branches were only for different accessions of individual species (*S. ovalis*, *S. parryoides*, *S. inopinata*, *S. americana*).

The microsatellite analysis provided a final matrix of 107 individuals with 37 variable markers. The accessions of the outgroup taxa *S. czukotica* and *S. altaica* were excluded from

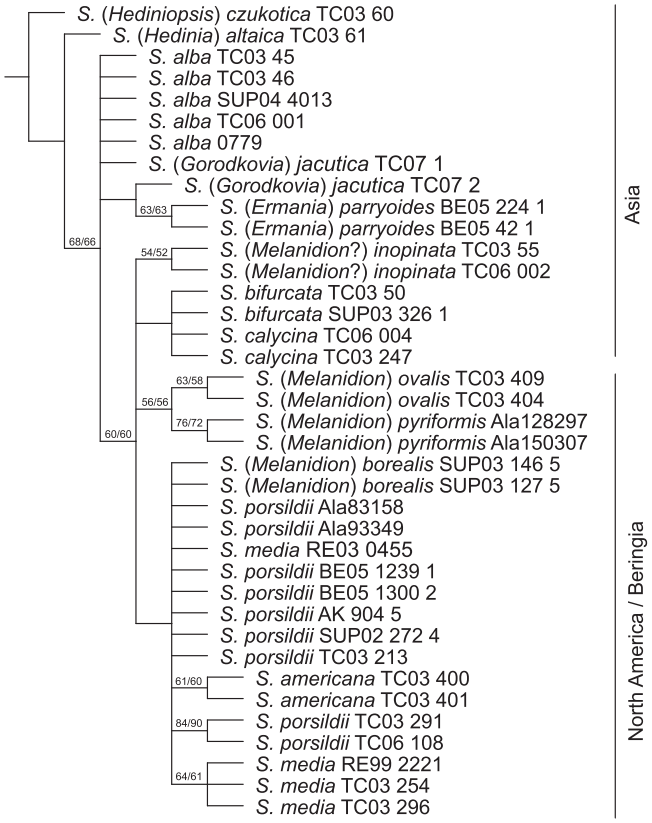

Fig. 4. Strict consensus tree of 1365 most parsimonious trees from an analysis of combined plastid sequences from the *trnL*^{UAA} intron, the *trnL*^{UAA}-*trnF*^{GAA} intergenic spacer region, the *rps16* intron, the *trnH*^{GUG}-*psbA* intergenic spacer, and the 5′*prs12-rpl20* intergenic spacer. Numbers above branches are jackknife/bootstrap support values. Numbers below branches are Bremer support values.

the final analysis as they only yielded private markers for most primers. In the PCO plot, axis 1 and 2 explained 22.2% and 17.9% of the variation, respectively (Fig. 5). The STRUCTURE analysis with $K = 3$ separated the accessions into three groups, which also could be recognized in the PCO plot. One group comprised the species formerly assigned to the genera *Ermania*, *Gorodkovia*, and *Melanidion*. The remaining two groups comprised the species of *Smelowskia* s.str., one with the Asian (non-Beringian) species (*S. alba*, *S. bifurcata*, *S. calycina*) and one with the American/Beringian species (*S. americana*, *S. media*, *S. porsildii*). In a separate PCO analysis of the latter group (Fig. 6), *S. americana*, *S. media* and *S. porsildii* could be separated along the first two axes. The accessions initially referred to *S. jurtzevii* and *S. spathulatifolia* grouped together with *S. porsildii* in this plot, and could neither be separated on any of the next eight axes in the PCO analysis nor in STRUCTURE.

As expected, some accessions of the species reported as polyploid had microsatellite profiles incompatible with diploidy at some loci (AthCTRI, ATTS0191, nga1145, IS-17). These profiles contained three or four different alleles (indicated in Table S1, see Electronic Supplement). All accessions but one of *S. americana* and 11 out of 39 accessions of *S. porsildii* showed

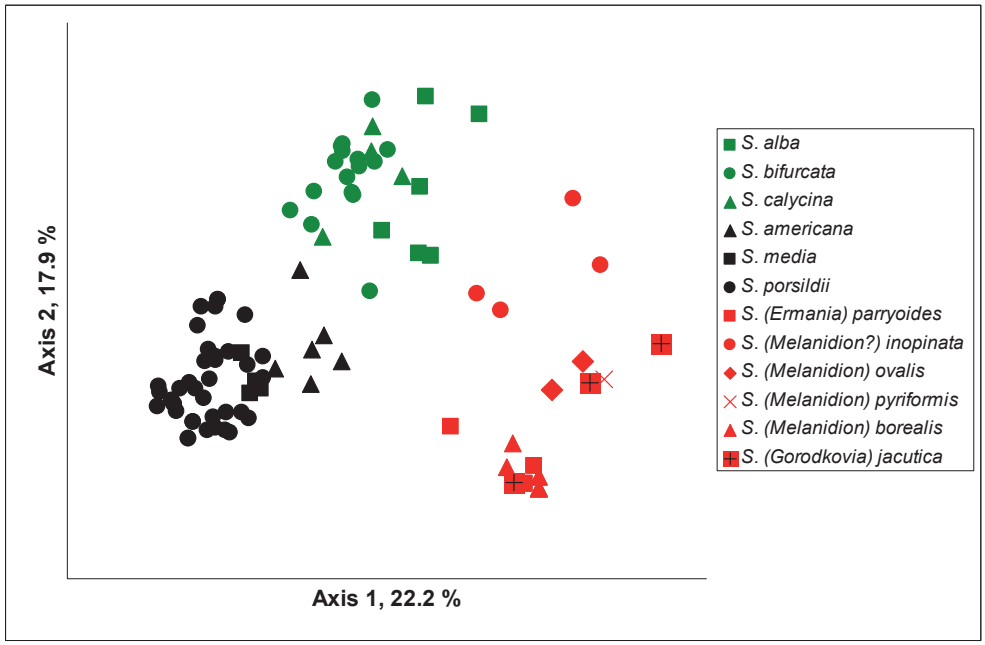

Fig. 5. Principal coordinate analysis plot based on the microsatellite dataset. The colours identify the different genetic groups recognized in the STRUCTURE analysis: black, Beringian and American species of *Smelowskia* s.str.; green, Asian (non-Beringian) species of *Smelowskia* s.str.; red,species formerly referred to *Melanidion*, *Ermania*, and *Gorodkovia*.

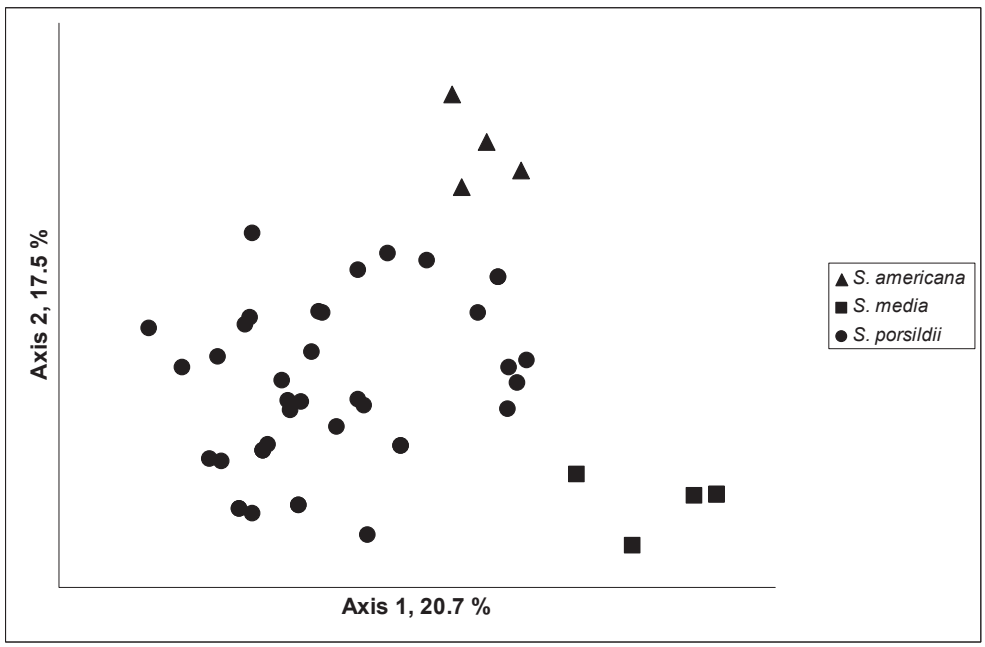

Fig. 6. Separate principal coordinate analysis plot of the Beringian and American species of *Smelowskia* s.str. based on the microsatellite dataset.

a polyploid profile at one or more of these four loci. Most accessions of the species reported as diploid had microsatellite profiles compatible with diploidy at all seven loci. However, 6 out of 17 accessions of *S. bifurcata*, which previously only has been reported as diploid, showed a polyploid profile at the locus AthCTRI. All alleles present in the six specimens with polyploid profiles were also found in the eleven specimens with diploid profiles, suggesting that the alleles were homologous.

■ DISCUSSION

This study has shown that microsatellite analysis in combination with sequencing of several nuclear regions provide a powerful approach to resolve relationships in recently evolved genera, which are frequently encountered in the arctic flora. This is particularly important for groups occurring in inaccessible areas from which fresh material for DNA fingerprinting is difficult to obtain. We observed only little cpDNA variation in *Smelowskia*, as previously found in other arctic plant groups such as in *Draba* and *Cardamine* L. (Koch & Al-Shehbaz, 2002; Carlsen & al., 2009). This corroborates the hypothesis that many arctic species have formed recently, probably during the Pleistocene (Carlsen, 2007; Carlsen & al., 2009). We found no indications that our cloning approach amplified different homeologs of low copy nuclear sequences, which otherwise could have indicated allopolyploidy from divergent progenitors or incomplete lineage sorting, as have been seen in some other studies of recently diverged and/or polyploid lineages (Kovarik & al., 2005; Brysting & al., 2007; Eidesen & al., 2007a). As in Popp & Oxelman (2004) we found no trace of any gene duplication event prior to speciation in our *RPA2* dataset, indicating that this region is a single copy region in *Smelowskia*.

The *Smelowskia* clade – one or several genera? — Our molecular analyses of the *Smelowskia* clade shows a structure corresponding to the former subdivision of this clade into several genera. The analyses based on ITS, *RPA2* and SSRs all identify *Smelowskia* s.str. as a group separate from *Melanidion* and *Ermania*. The results of the two nuclear regions and the SSRs are congruent and separate *Smelowskia* s.str. from *Melanidion*. With an assignment of *S. inopinata* to *Melanidion*, the old genus subdivision thus seems to be justified by the SSR and *RPA2* datasets. The ITS dataset also suggests *Ermania* as sister to the *Melanidion*/*Smelowskia* clade, and *Redowskia* as sister to this group.

Within *Smelowskia* s.str. there is a geographic separation of the Old World and New World (including Beringian) accessions in the SSRs, which is not contradicted by the sequence-based phylogenies. Based on the chloroplast phylogeny, however, it would seem that American accessions of *Smelowskia* s.str. and *Melanidion* constitute a separate group originating from Asian ancestors. This does not fit well with morphological and nuclear molecular data. These incongruent results could be explained by horizontal transfer of the plastid genome, e.g., a hybridization event between *S. borealis* and *S. alba*. Such incongruences between nuclear and plastid genomes have frequently been reported in plants (Rieseberg & Soltis, 1991; Rieseberg & al., 1996; Petit & al., 2004). However, the support for such a scenario is weak in the plastid tree, and is contradicted by the SSRs (Fig. 5).

There are two solutions to the problem of genus delimitation. One solution is to lump all species together into one large polymorphic genus, as suggested by Al-Shehbaz & Warwick (2006). The other is to maintain *Smelowskia* s.str. and the satellite genera *Ermania*, *Melanidion*, *Gorodkovia*, and *Redowskia*. This solution also implies exclusion from *Smelowskia* of the additional satellite genera which fell outside the *Smelowskia* clade as defined in this study (*Hedinia*, *Hediniopsis*, *Sophiopsis*, *Sinosophiopsis*), and that *S. inopinata* is assigned to *Melanidion*. However, as we have not investigated all taxa in this study, we will recommend retaining one large genus until supplementary studies are performed.

Number and delimitation of species. — Most of the species we tentatively recognized in the initial framework for this study seem to be adequately separable by molecular markers. However, we note that *S. bifurcata* and *S. calycina*, easily separable based on calyx and siliqula characters (Ovchinnikova, 2004) are barely separable by the markers studied here. Based on their clear morphological differences, we suggest to keep these as two separate species. We also found that accessions of *S. alba* from the Lena River delta differ somewhat from those from the Altai Mountains. This molecular difference may be ascribed to divergence between two disjunct distribution areas which has not yet resulted in morphological divergence with taxonomic implications. Although the accessions of *S. media* and *S. porsildii* were inadequately separated in all the sequence analyses, they were fairly distinct based on SSRs. Their distinction is also supported by their different leaf shape; *S. media* has pinnately lobed basal and cauline leaves, whereas *S. porsildii* has leaves that are entire or with shallow apical teeth (Drury & Rollins, 1952).

The SSR data provided further evidence of the importance of polyploidization in this arctic-alpine group. *Smelowskia bifurcata* was reported as diploid with $2n = 12$ by Krogulevich (1976), but we found more than two alleles per individual at the locus AthCTRI. This may be due to a single-region duplication, but it is more likely that there are multiple cytotypes also within this species as there were no "new" alleles in the tetraploid profiles. Another possible explanation is misidentification of the specimens that are counted. Krogulevich (1976) counted specimens from the Putoran Plateu (Lake Bogatyr), which is outside of the commonly recognized distribution area of *S. bifurcata*, but within the range of the morphologically similar *S. alba*, which has been reported as diploid (Ovchinnikova, 2004). More chromosome counts are clearly necessary for *S. bifurcata* as well as other species of *Smelowskia*.

Our results suggest that *S. porsildii* should be recognized as one variable species, including *S. spathulatifolia* and *S. jurtzevii*. We found no molecular differences between the accessions referred to them, and there are apparently no good morphological distinguishing characters (Warwick & al., 2004; Al-Shehbaz & Warwick, 2006). The name *Smelowskia porsildii* (W.H. Drury & Rollins) Jurtzev has priority (Jurtzev, 1970), and *Smelowskia spathulatifolia* Velichkin and *Smelowskia*

jurtzevii Velichkin should be treated as synonyms. For a full list of synonyms see Velichkin (1974, 1979).

The Beringian species *S. porsildii* does not show a close relationship with the Asian (non-Beringian) taxa, but rather with the American *S. media* and *S. americana*. These three species are allopatric, occurring in three separate areas left unglaciated during the Wisconsian maximum (Fig. 1; Dyke, 2004). It is thus possible that they result from divergent speciation in different glacial refugia.

Origin and spread of the *Smelowskia* clade. — Based on its sister group relationships, it is most likely that the *Smelowskia* clade originated in Central Asia. Except for *S. czukotica*, all species outside what we have called the *Smelowskia* clade are Central Asian. The American and Beringian representatives within the clade are restricted to two phylogenetic lineages, whereas the Asian representatives are spread throughout the tree. If the clade originated in Asia, only two dispersal events from Asia to North America have to be inferred. Alternatively, a North American origin would necessitate at least four dispersal events. Thus, our results are not consistent with a North American origin of the group (from the *S. media* lineage) as suggested by Drury & Rollins (1952). They only studied the North American representatives, but looking at this region separately, there is still no indication from our study that *S. media* has any key role in the origin of the North American taxa.

■ ACKNOWLEDGEMENTS

We thank all institutions and colleagues (cf. Table S1) for providing plant material for our study. Some material was obtained via R. Elven's participation in the Tundra North-West (TNW) '99 expedition funded by the Polar Research Secretariat at the Royal Swedish Academy of Sciences. I. Skrede, M.H. Jørgensen and M. Popp are thanked for helpful comments. We thank N. Kane for searching the *A. thaliana* genome for IS-17. This study was funded as part of the Strategic University Programme grant 146515/420 from the Research Council of Norway to C. Brochmann and R. Elven (subprogramme Migration and Evolution of Arctic Plants in Response to Quaternary Climate Changes).

■ LITERATURE CITED

Abbott, R.J. & Brochmann, C. 2003. History and evolution of the arctic flora: In the footsteps of Eric Hultén. *Molec. Ecol.* 12: 299–313.

Al-Shehbaz, I.A. 2003. (1583) Proposal to conserve the name *Smelowskia* against *Redowskia* (Brassicaceae). *Taxon* 52: 360.

Al-Shehbaz, I.A., Beilstein, M.A. & Kellogg, E.A. 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): An overview. *Pl. Syst. Evol.* 259: 89–120.

Al-Shehbaz, I.A. & Warwick, S.I. 2006. A synopsis of *Smelowskia* (Brassicaceae). *J. Arnold Arbor.* 11: 91–99.

Bennike, O. & Böcher, J. 1990. Forest-tundra neighboring the North Pole—Plant and insect remains from the Plio-Pleistocene Kap København formation, North Greenland. *Arctic* 43: 331–338.

Bremer, K. 1994. Branch support and tree stability. *Cladistics* 10: 295–304.

Brochmann, C. & Brysting, A.K. 2008. The Arctic—An evolutionary freezer? *Pl. Ecol. Div.* 1: 181–195.

Brochmann, C., Brysting, A.K., Alsos, I.G., Borgen, L., Grundt, H.H., Scheen, A.-C. & Elven, R. 2004. Polyploidy in arctic plants. *Biol. J. Linn. Soc.* 82: 521–536.

Brummitt, R.K. 2005. Report of the committee for spermatophyta: 56. *Taxon* 54: 527–536.

Bruvo, R., Michiels, N.K., D'Souza, T.G. & Schulenburg, H. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molec. Ecol.* 13: 2101–2106.

Brysting, A.K., Fay, M.F., Leitch, I.J. & Aiken, S.G. 2004. One or more species in the arctic grass genus *Dupontia*?—A contribution to the Panarctic Flora project. *Taxon* 53: 365–382.

Brysting, A.K., Oxelman, B., Huber, K.T., Moulton, V. & Brochmann, C. 2007. Untangling complex histories of genome mergings in high polyploids. *Syst. Biol.* 56: 467–476.

Carlsen, T. 2007. *Are there any trees in the Arctic? Reconstruction of evolutionary histories in a young biome*. Ph.D. Thesis, University of Oslo.

Carlsen, T., Bleeker, W., Hurka, H., Elven, R. & Brochmann, C. 2009. Biogeography and phylogeny of *Cardamine* (Brassicaceae). *Ann. Missouri Bot. Gard.* 96: 215–236.

Czerepanov, S.K. 1995. *Vascular plants of Russia and adjacent states (the former USSR)*. Cambridge: Cambridge Univ. Press.

Dawe, J.C. & Murray, D.F. 1979. P. 266 in: Löve, Á. (ed.), IOPB Chromosome number reports. LXIII. *Taxon* 28: 265–279.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. *Ecology* 26: 297.

Drury, W.H. & Rollins, R.C. 1952. The North American representatives of *Smelowskia* (Cruciferae). *Rhodora* 54: 85–119.

Dyke, A.S. 2004. An outline of North American deglaciation with emphasis on central and northern Canada. Pp. 373–424 in: Ehlers, J. & Gibbard, P.L. (eds.), *Quaternary glaciations: Extent and chronology*. Amsterdam: Elsevier.

Eidesen, P.B., Alsos, I.G., Popp, M., Stensrud, Ø., Suda, J. & Brochmann, C. 2007a. Nuclear vs. plastid data: Complex Pleistocene history of a circumpolar key species. *Molec. Ecol.* 16: 3902–3925.

Eidesen, P.B., Carlsen, T., Molau, U. & Brochmann, C. 2007b. Repeatedly out of Beringia: *Cassiope tetragona* embraces the Arctic. *J. Biogeogr.* 34: 1559.

Farris, J.S. 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417–419.

Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D. & Kluge, A.G. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* 27: 401–410.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 4: 783–791.

Geml, J., Laursen, G.A., O'Neill, K., Nusbaum, H.C. & Taylor, D.L. 2006. Beringian origins and cryptic speciation events in the fly agaric (*Amanita muscaria*). *Molec. Ecol.* 15: 225–239.

Goldstein, D.B. & Pollock, D.D. 1997. Launching microsatellites: A review of mutation processes and methods of phylogenetic inference. *J. Heredity* 88: 335–342.

Goloboff, P.A., Farris, J.S. & Nixon, K.C. 2008. TNT: A free program for phylogenetic analysis. *Cladistics* 24: 774–786.

Grundt, H.H., Kjølner, S., Borgen, L., Rieseberg, L.H. & Brochmann, C. 2006. High biological species diversity in the arctic flora. *Proc. Natl. Acad. Sci. U.S.A* 103: 972–975.

Grundt, H.H., Popp, M., Brochmann, C. & Oxelman, B. 2004. Polyploid origins in a circumpolar complex in *Draba* (Brassicaceae) inferred from cloned nuclear DNA sequences and fingerprints. *Molec. Phylog. Evol.* 32: 695.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41: 95–98.

Hamilton, M.B. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molec. Ecol.* 8: 521–523.

Harr, B., Zangerl, B., Brem, G. & Schlotterer, C. 1998. Conservation of locus-specific microsatellite variability across species: A comparison of two *Drosophila* sibling species, *D. melanogaster* and *D. simulans*. *Molec. Biol. Evol.* 15: 176–184.

Hewitt, G.M. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philos. Trans., Ser. B* 359: 183.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Jahren, A.H. 2007. The Arctic Forest of the Middle Eocene. *Annual Rev. Earth Planet. Sci.* 35: 509–540.

Johnson, A.W. & Packer, J.G. 1968. Chromosome numbers in the flora of Ogotoruk Creek, N.W. Alaska. *Bot. Not.* 121: 403–456.

Jørgensen, M.H., Carlsen, T., Skrede, I. & Elven, R. 2008. Microsatellites resolve the taxonomy of the polyploid *Cardamine digitata* aggregate (Brassicaceae). *Taxon* 57: 882–892.

Jørgensen, M.H., Elven, R., Tribsch, A., Gabrielsen, T.M., Stedje, B. & Brochmann, C. 2006. Taxonomy and evolutionary relationships in the *Saxifraga rivularis* complex. *Syst. Bot.* 31: 702–729.

Jurtzev, B. 1970. De plantarum speciebus novis e terra Tschuktschorum. *Novosti Sist. Vyssh. Rast.* 6: 302–320.

Kluge, A.G. & Farris, J.S. 1969. Quantitative phyletics and evolution of Anurans. *Syst. Zool.* 18: 1–32.

Koch, M. & Al-Shehbaz, I.A. 2002. Molecular data indicate complex intra- and intercontinental differentiation of American *Draba* (Brassicaceae). *Ann. Missouri Bot. Gard.* 89: 88–109.

Kovarik, A., Pires, J.C., Leitch, A.R., Lim, K.Y., Sherwood, A.M., Matyasek, R., Rocca, J., Soltis, D.E. & Soltis, P.S. 2005. Rapid concerted evolution of nuclear ribosomal DNA in two *Tragopogon* allopolyploids of recent and recurrent origin. *Genetics* 169: 931–944.

Krogulevich, R.E. 1976. Rol poliploidii v genezise flory Putorana. Pp. 217–235 in: Malyschev, L.I. (ed.), *Flora Putorana. Materialy k poznaniiu osobennostei sostava i genezica gornykh sybarkticheskikh flor Sibiri*. Novosibirsk: Izdatelstvo "Nauka".

Lafontaine, J.D. & Wood, D.M. 1988. A zoogeographic analysis of the Noctuidae (Lepidoptera) of Beringia, and some inferences about past Beringian habitats. *Mem. Entomol. Soc. Canada* 144: 109–123.

Lear, C.H., Elderfield, H. & Wilson, P.A. 2000. Cenozoic deep-sea temperatures and global ice volumes from Mg/Ca in benthic foraminiferal calcite. *Science* 287: 269–272.

Matthews, J.V. & Ovenden, L.E. 1990. Late Tertiary plant macrofossils from localities in arctic/sub-arctic North-America—A review of the data. *Arctic* 43: 364–392.

Mulligan, G.A. 2001. Three new taxa and a summary of the mustard family, Brassicaceae (Cruciferae), in Canada and Alaska. *Canad. Field-Naturalist* 115: 341–342.

Murray, D.F. 1995. Causes of arctic plant diversity: Origin and evolution. *Ecol. Stud.* 113: 21–32.

Murray, D.F. & Kelso, S. 1997. Chromosome numbers and notes on the taxonomy of selected Alaskan vascular plants. *Rhodora* 99: 33–55.

Nylander, J.A.A. 2004. MrModeltest, version 2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University.

Ovchinnikova, S.V. 2004. Family Brassicaceae, Genera *Macropodium* R. Br.—*Gorodkovia* Botch. et Karav. Pp. 45–63 in: Malyschev, L.I. (ed.), *Flora of Siberia*. Plymouth: Science Publishers Inc.

Oxelman, B., Liden, M. & Berglund, D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Pl. Syst. Evol.* 206: 393–410.

Packer, J.G. 1968. P. 287 in: Löve, Á. (ed.), IOPB Chromosome number reports XVII. *Taxon* 17: 285–288.

Petit, R.J., Bodenes, C., Ducousso, A., Roussel, G. & Kremer, A. 2004. Hybridization as a mechanism of invasion in oaks. *New Phytol.* 161: 151–164.

Popp, M., Erixon, P., Eggens, F. & Oxelman, B. 2005. Origin and evolution of a circumpolar polyploid species complex in *Silene* (Caryophyllaceae) inferred from low copy nuclear RNA polymerase introns, rDNA, and chloroplast DNA. *Syst. Bot.* 30: 302–313.

Popp, M. & Oxelman, B. 2004. Evolution of a RNA polymerase gene family in *Silene* (Caryophyllaceae)—Incomplete concerted evolution and topological congruence among paralogues. *Syst. Biol.* 53: 914–932.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Rieseberg, L.H. & Soltis, D.E. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evol. Trends Pl.* 5: 65–84.

Rieseberg, L.H., Whitton, J. & Klinder, C.R. 1996. Molecular marker incongruence in plant hybrid zones and phylogenetic trees. *Acta Bot. Neerl.* 45: 243–262.

Rohlf, F.J. 1990. Morphometrics. *Annual Rev. Ecol. Syst.* 21: 299.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Rydberg, P.A. 1902. Studies on the Rocky Mountain flora—VIII. *Bull. Torrey Bot. Club* 29: 232–250.

Sang, T., Crawford, D.J. & Stuessy, T.F. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *Amer. J. Bot.* 84: 1120–1136.

Scheen, A.-C., Brochmann, C., Brysting, A.K., Elven, R., Morris, A., Soltis, D.E., Soltis, P.S. & Albert, V.A. 2004. Northern Hemisphere biogeography of *Cerastium* (Caryophyllaceae): Insights from phylogenetic analysis of noncoding plastid nucleotide sequences. *Amer. J. Bot.* 91: 943–952.

Scheen, A.-C., Elven, R. & Brochmann, C. 2002. A molecular-morphological approach solved taxonomic controversy in arctic *Draba* (Brassicaceae). *Canad. J. Bot.* 80: 59–71.

Schlötterer, C. 2001. Genealogical inference of closely related species based on microsatellites. *Genet. Res.* 78: 209–212

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nat. Biotechnol.* 18: 233–234.

Shavit, L., Penny, D., Hendy, M.D. & Holland, B.R. 2007. The Problem of rooting rapid radiations. *Molec. Biol. Evol.* 24: 2400–2400.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Amer. J. Bot.* 92: 142–166.

Skrede, I., Carlsen, T., Rieseberg, L.H. & Brochmann, C. 2009. Microsatellites for three distantly related genera in the Brassicaceae. *Cons. Genet.* 10: 643–648.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of 3 noncoding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

Tkach, N.V., Hoffmann, M.H., Röser, M., Korobkov, A.A., von Hagen, K.B. & Feder, J. 2008. Parallel evolutionary patterns in multiple lineages of arctic *Artemisia* L. (Asteraceae). *Evolution* 62: 184–198.

Velichkin, E.M. 1974. On the new species from the genus *Smelowskia* C.A. Mey. (Cruciferae) in the south-east of the U.S.S.R. *Bot. Zhurn. (Moscow & Leningrad)* 59: 1805–1808.

Velichkin, E.M. 1979. *Smelowskia* C.A. Mey. (Cruciferae): Critical review and relation to close genera. *Bot. Zhurn. (Moscow & Leningrad)* 64: 153–171.

Warwick, S.I., Al-Shehbaz, I.A., Sauder, C.A., Murray, D.F. & Mummenhoff, K. 2004. Phylogeny of *Smelowskia* and related genera (Brassicaceae) based on nuclear ITS DNA and chloroplast *trnL* intron DNA sequences. *Ann. Missouri Bot. Gard.* 91: 99–123.

Weider, L.J. & Hobæk, A. 2000. Phylogeography and arctic biodiversity: A review. *Ann. Zool. Fenn.* 37: 217–231.

White, T., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and

direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M.A., Gelfand, D.H., Sninski, J.J. & White, T.J. (eds.), *PCR-protocols: A guide to methods and applications*. San Diego: Academic press.

Yurtsev, B.A. & Zhukova, P.G. 1972. Cytotaxonomical characteristics of endemic plants of mountainous North-East of Asia. *Bot. Zhurn. (Moscow & Leningrad)* 57: 50–63.

Yurtsev, B.A. & Zhukova, P.G. 1982. Chromosome numbers of some plants of the Northeastern Yakutia (The drainage of the Indigirka River in its middle reaches). *Bot. Zhurn. (Moscow & Leningrad)* 67: 788–787.

Yurtsev, B.A., Zhukova, P.G., Plieva, T.V., Raszhivin, V.Y. & Sekretareva, N.A. 1975. Interesting floristic finds in the easternmost Chukotka peninsula. III. *Bot. Zhurn. (Moscow & Leningrad)* 60: 233–247.

Zhukova, P.G. 1980. Chromosome numbers of some southern Chukotka plant species. *Bot. Zhurn. (Moscow & Leningrad)* 65: 51–59.

Zhukova, P.G. & Petrovsky, V.V. 1977. Chromosome numbers in some western Chukotka plant species. III. *Bot. Zhurn. (Moscow & Leningrad)* 62: 1215–1223.

Zhukova, P.G. & Petrovsky, V.V. 1980. Chromosome numbers and taxonomy of some species of the Anyui mts. *Bot. Zhurn. (Moscow & Leningrad)* 65: 651–659.

Zhukova, P.G. & Petrovsky, V.V. 1984. A cytotaxonomical study of some species of the family Brassicaceae in northeastern Asia. *Bot. Zhurn. (Moscow & Leningrad)* 69: 236–240.